Molecular Biobricking

Methods of the iGEM Lab TU Darmstadt

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Content

Introduction

Working on the last years' iGEM projects resulted in a variety of protocols and recipes which were collected and sorted for creating "Molecular BioBricking – an iGEM lab manual". "Molecular BioBricking" is meant to help rookies to get started and affords an opportunity for advanced iGEMers to look up tricky procedures.

As the name may suggest, most protocols refer to "Molecular Cloning: A lab manual" (*1*). To enlarge the spectrum of methods, protocols of several publications and utilized kits and were additionally added.

Feel free to share this lab manuals to facilitate your wetlab efforts.

Best wishes,

iGEM Team TU Darmstadt 2015

In vitro

1. DNA

Agarose gel electrophoresis

Short explanation

Agarose gel electrophoresis is the most commonly used method to separate nucleic acids. Due to their negative charge, DNA and RNA molecules can be moved through an agarose gel by an electric field (electrophoresis). Longer molecules move slower through the agarose matrix while short ones move faster and thus migrate further.

Procedure

In common 0.8% to 2% agarosegels were used. Low concentrated gels lead to better results for large DNA fragments (2-6 kbp), while high concentrated gels lead to better results for small DNA fragments (0.3-0.7 kbp).

1. Mix desired amount of agarose with ddH_2O
2. Heat up liquid in microwave until whole agarose is solved

Let liquid cool down until one can touch the bottle by Hand. Mix 5µL Nancy-520 with 50mL agarose gel.

3. Fill mixture into gel chamber and let it cool down (do not forget the well combs)
4. Fill up chamber with 1xTAE-Buffer
5. Take off well comb
6. Pipette 3-4 µL DNA ladder of choice into first pocket
7. Mix samples 5:1 with 6x loading dye (5 µL sample with 1 µL loading dye) and pipette into pockets
8. Run electrophoresis at 120 V

Dephosphorylation

Short explanation

Antarctic Phosphatase catalyzes the removal of 5' phosphate groups of DNA and RNA and thus prevents religation of cut vectors. It is applied before ligation.

Procedure

1. Reaction Mix

- Restriction product
- 1/10 of reaction end volume 10x Antarctic Phosphatase Reaction Buffer
- 1 µL of Antarctic Phosphatase

2. Incubate at 37° C for 30 minutes
3. Heat inactivate at 70° C for 5 minutes
4. Continue with ligation

DNA Quantification via NanoDrop

Materials & Equipment

- Micropipettes with sterile tips
- Nanodrop

Chemicals & consumables

- DNA sample
- Water

Procedure

1. Start the NanoDrop program and choose "Nucleic Acids"

2. Pipette a 1 µL water sample onto the sample holder and click “OK” for initializing

3. Load your blank and click "Blank"

4. Load your DNA samples and click "Measure"

The system calculates automatically 260/280 and 230/260 values.

DNA Ligation

Short explanation

DNA ligation is necessary to assemble digested DNA parts into a vector. The cut ends generated by restriction enzymes are put together by DNA ligase.

Procedure - Ligation (20 µL batch)

1. Reaction mixture
    - T4 Ligase Buffer 2 µL
    - T4 Ligase 1 µL
    - Digested Insert 6 µL
    - Digested backbone 2 µL Molar ratio: 3:1 (Insert: Vector)
    - add ddH_2O to 20µL end volume
2. Incubate at room temperature for 30 min (Alternatively incubate at 16°C overnight)
3. Inactivate T4 ligase at 70°C for 10 min

PCR

Short report

PCR (polymerase chain reaction) is a method for exponentially amplifying a fragment of DNA in vitro. Pfu polymerase is a cheap and precise alternative to expensive “high-end” polymerases like Q5 and Phusion.

Materials & Equipment

- Micropipettes with sterile tips
- Thermocycler
- PCR tubes

Chemicals & consumables	***50 µL***
• Pfu Mastermix 9:25	18 µL
• DNA template	~100 pg (1 µL of a 1:100 template dilution)
• Forward primer (10 µM)	1 µL
• Reverse primer (10 µM)	1 µL
• ddH_2O	fill volume up to 50 µL

Procedure

Standard protocol for Pfu Polymerase

#	Temperature	Time
1	95°C	00:02:00
2	95°C	00:00:10
3	55 °C	00:00:30
4	72 °C	2 min/kbp
5	GO TO 2	REPEAT 30x
6	72 °C	3 min/kbp
7	4 °C	HOLD

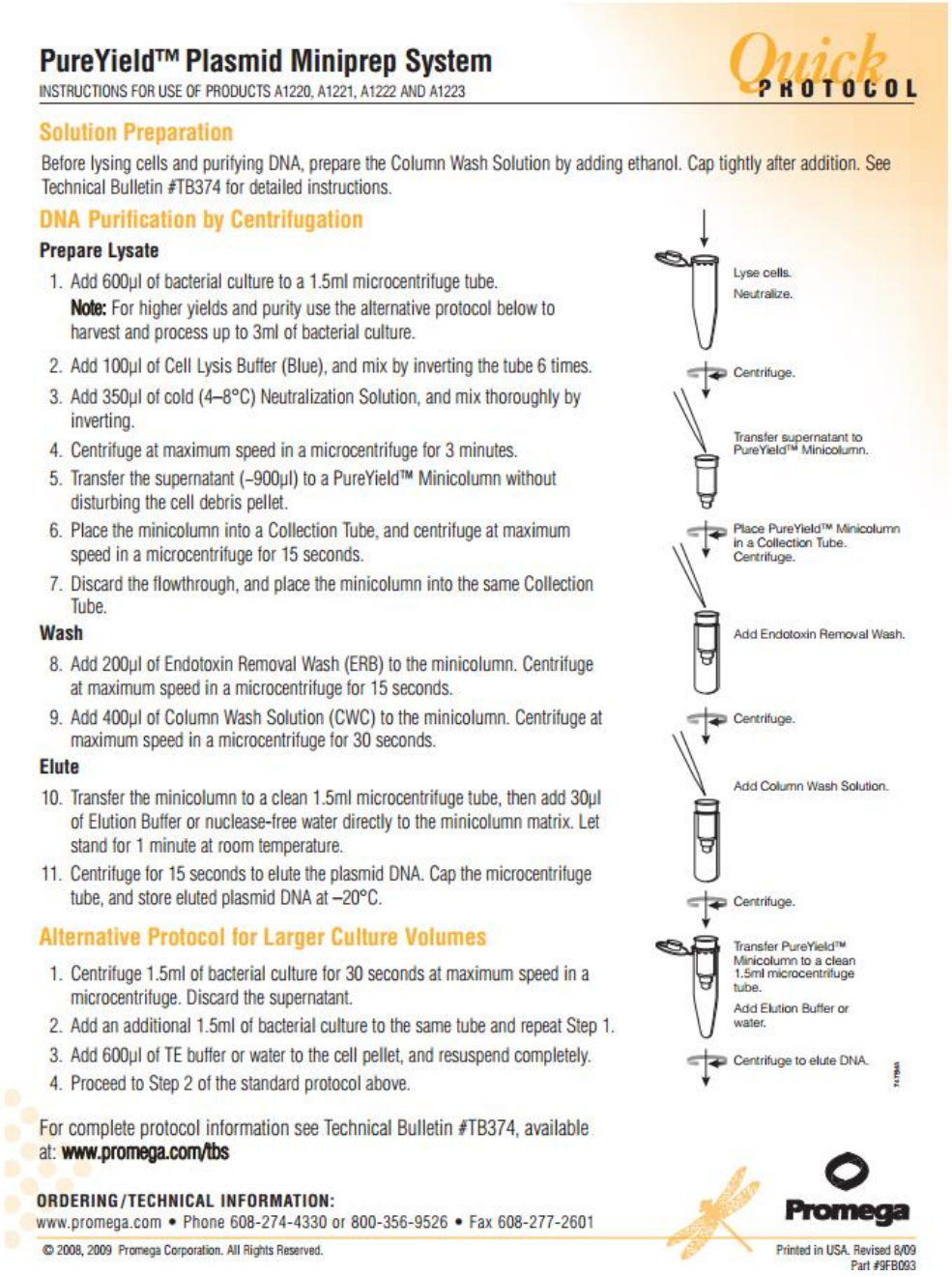
Taq polymerase, another cheap alternative, uses extension temperature of 68°C and performs with 1 kbp/min. Taq polymerase lacks an exonuclease proofreading. In consequence the Taq polymerase is less precise and it is used for analytical PCRs (*e.g.* Colony PCR screening).

Plasmid Preparation

Short explanation

Plasmids can be prepared from cell culture by chemical precipitation. The PureYield Plasmid Miniprep System Kit by Promega offers a fast way to purify plasmid DNA from bacteria cultures.

Procedure



PureYield™ Plasmid Miniprep System

INSTRUCTIONS FOR USE OF PRODUCTS A1220, A1221, A1222 AND A1223

Quick PROTOCOL

Solution Preparation

Before lysing cells and purifying DNA, prepare the Column Wash Solution by adding ethanol. Cap tightly after addition. See Technical Bulletin #TB374 for detailed instructions.

DNA Purification by Centrifugation

Prepare Lysate

1. Add 600µl of bacterial culture to a 1.5ml microcentrifuge tube.
   Note: For higher yields and purity use the alternative protocol below to harvest and process up to 3ml of bacterial culture.
2. Add 100µl of Cell Lysis Buffer (Blue), and mix by inverting the tube 6 times.
3. Add 350µl of cold (4–8°C) Neutralization Solution, and mix thoroughly by inverting.
4. Centrifuge at maximum speed in a microcentrifuge for 3 minutes.
5. Transfer the supernatant (~900µl) to a PureYield™ Minicolumn without disturbing the cell debris pellet.
6. Place the minicolumn into a Collection Tube, and centrifuge at maximum speed in a microcentrifuge for 15 seconds.
7. Discard the flowthrough, and place the minicolumn into the same Collection Tube.

Wash

8. Add 200µl of Endotoxin Removal Wash (ERB) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 15 seconds.
9. Add 400µl of Column Wash Solution (CWC) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 30 seconds.

Elute

10. Transfer the minicolumn to a clean 1.5ml microcentrifuge tube, then add 30µl of Elution Buffer or nuclease-free water directly to the minicolumn matrix. Let stand for 1 minute at room temperature.
11. Centrifuge for 15 seconds to elute the plasmid DNA. Cap the microcentrifuge tube, and store eluted plasmid DNA at –20°C.

Alternative Protocol for Larger Culture Volumes

1. Centrifuge 1.5ml of bacterial culture for 30 seconds at maximum speed in a microcentrifuge. Discard the supernatant.
2. Add an additional 1.5ml of bacterial culture to the same tube and repeat Step 1.
3. Add 600µl of TE buffer or water to the cell pellet, and resuspend completely.
4. Proceed to Step 2 of the standard protocol above.

For complete protocol information see Technical Bulletin #TB374, available at: **www.promega.com/tbs**

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For higher yields, the elution buffer (EB or H_2O) can be pre-heated to 60-70°C or sample can be incubated for 5 min instead of 1 min.

Restriction digest

Short explanation

In order to insert DNA fragments into plasmids via ligation it is necessary to digest both components with restriction enzymes. Most restriction enzymes need a 3-4 bp overhang. This must be considered when designing primers for inserts.

Procedure - Single DNA Digestion (50 µL batch)

The following is an example of a typical analytical single restriction enzyme digestion with NEB restriction enzymes.

1. Add up the following:
    - 500 ng DNA
    - 5 µL 10x appropriate enzyme buffer
    - 1 µL restriction enzyme (10 u)
    - Fill up to 50 µL with nuclease free H_2O
2. Incubate for 30 min at 37°C (read supplier's information)
3. Inactivate enzymes by incubating at 80°C for 20 min (read supplier's information)

Some enzymes can be heat-inactivated at lower temperatures. Some cannot be heat-inactivated at all (like BamHI) and have to be removed with the PCR clean-up kit

Larger or smaller scale DNA digestions can be accomplished by scaling this basic reaction proportionately.

Procedure - Multiple Restriction Enzyme Digests (50 µL batch)

Use the optimal buffer supplied with one enzyme if the activity of the second enzyme is acceptable in that same buffer (Check table supplied by NEB, BioBrick Assembly: Buffer 2.1). Follow the single restriction enzyme digestion by using 1 µL of the additional enzyme and take 1 µL less of nuclease-free water.

PCR Clean Up

Equipment

- PCR Clean Up Kit (*e.g.* Wizard® SV Gel and PCR Clean-Up Kit)
- centrifuge
- 1.5 mL cap tubes

Procedure

- Reference Wizard® SV Gel and PCR Clean-Up System Quick Protocol by Promega

Wizard® SV Gel and PCR Clean-Up System

INSTRUCTIONS FOR USE OF PRODUCTS A9280, A9281, A9282, AND A9285.

Quick PROTOCOL

DNA Purification by Centrifugation

Gel Slice and PCR Product Preparation

A. Dissolving the Gel Slice

1. Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5ml microcentrifuge tube.
2. Add 10µl Membrane Binding Solution per 10mg of gel slice. Vortex and incubate at 50–65°C until gel slice is completely dissolved.

B. Processing PCR Amplifications

1. Add an equal volume of Membrane Binding Solution to the PCR amplification.

Binding of DNA

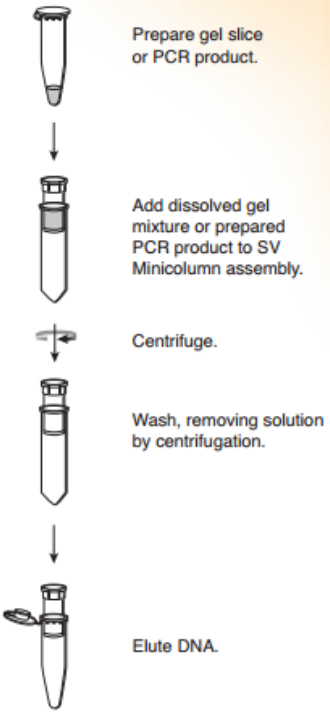
1. Insert SV Minicolumn into Collection Tube.
2. Transfer dissolved gel mixture or prepared PCR product to the Minicolumn assembly. Incubate at room temperature for 1 minute.
3. Centrifuge at 16,000 × *g* for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.

Washing

4. Add 700µl Membrane Wash Solution (ethanol added). Centrifuge at 16,000 × *g* for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
5. Repeat Step 4 with 500µl Membrane Wash Solution. Centrifuge at 16,000 × *g* for 5 minutes.
6. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.

Elution

7. Carefully transfer Minicolumn to a clean 1.5ml microcentrifuge tube.
8. Add 50µl of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at 16,000 × *g* for 1 minute.
9. Discard Minicolumn and store DNA at 4°C or –20°C.



Additional protocol information is available in Technical Bulletin #TB308, available online at:
www.promega.com

3760MA07_2A

For gel extraction: If possible run the samples on gels with an agarose concentration not higher than 0.8% (this reduces final salt concentrations). Dissolve the gel always at 50°C and add additional Binding Buffer if the dissolving takes too long. Let the dissolved gel cool down to RT for 30 - 60s (high temperatures reduce DNA binding to the column).

For PCR clean-up, the same principle applies as for the Miniprep: Higher temperature and letting stand for longer than 1 minute may increase yield significantly.

2. Proteins

Protein extraction via osmotic shock

For extract and purify overexpressed proteins from cell pellets, it is possible to shock cells with hypertonic sucrose solution to release proteins. (*2*).

TES Buffer: 50 mM HEPES, 1 mM EDTA, 20% sucrose

Procedure: Cells are harvested from bacterial culture and the cell pellet is resuspended in 1 mL TES Buffer. Cell suspension is incubated for 10 min on ice and subsequently centrifuged at 13.000 rpm in a table centrifuge for 20 min. Supernatant is safed as TES sample and the remaining pellet is resuspended in 1 mL ddH_2O to remove further proteins from cellular structures. Again, samples are centrifuged for 20 min at 13.000 rpm. The supernatant is saved as ddH_2O sample. Protein assays can be conducted with TES and ddH_2O sample.

SDS PAGE

Materials & Equipment

- Two glass plates
- Gel caster
- Comb
- VWR Power Source 300V
- Heat block

Chemicals & consumables

- SDS
- Rotiphorese® (30%)

- Tris HCl
- Glycine
- TEMED
- APS
- Aqua dest.
- Isopropyle alcohol
- Glycerine
- Beta-Mercaptoethanol
- Bromphenol blue
- Coomassie brilliant blue G250
- Coomassie brilliant blue R250
- Methanol
- Acetate (99%)

Buffers & gels

- Separation buffer (0.5 M Tris, 0.4% SDS, pH = 8.8)
- Stacking buffer (0.5 M Tris, 0.4% SDS, pH = 6.6)
- Running buffer (0.25 M Tris, 2 M glycine, 1% SDS , pH = 8.3)

- Separation gel 12.5% (5 ml Separation buffer, 6.25 ml Rotiphorese®, 3.75 ml Aqua dest., 30 µl TEMED, 30 µl APS (40%))
- Stacking gel 4% (3 ml Separation buffer, 1.33 ml Rotiphorese®, 5.67 ml Aqua dest.,20 µl TEMED, 20 µl APS (40%)

- 3x Sample buffer (65 mM Tris, 4% SDS, 20% glycerine, 10% beta-Mercaptoethanol, 1 tip of bromphenol blue, pH = 6.75)
- Staining buffer (0.5 g Coomassie brilliant blue G250 & R250 each, 100 ml methanol, 100 ml Aqua dest., 20 ml acetate)
- Destaining buffer (400 ml Aqua dest., 100 ml methanol, 30 ml acetate)

Procedure – Load & run

1. Prepare separating gel and fill it into the chamber

2. Pour 1 mL isopropyl alcohol on the top of the gel to destroy air bubbles and prevent dehydration
3. Discard the isopropyl alcohol and pour the prepared stacking gel after separating gel is polymerized
4. Stick in the comb
5. If not used immediately, store the gel in wet cloth (to prevent dehydration) at 4°C
6. If used immediately, remove comb when the gel is fully polymerized and place it into SDS PAGE chamber
7. Fill up chamber with running buffer
8. Heat up samples with 3x sample buffer at 70°C for 15 min and apply 20 µl to each pocket
9. Load one pocket with a commercial protein marker
10. Start PAGE by applying 20 mA while stacking
11. Apply 40 mA during separation

Procedure - Staining & washing of gel

1. Disconnect glass plates containing the gel that has completed the run
2. Cut off stacking gel
3. Put separation gel into staining buffer and shake at room temperature for at least one hour
4. Put stained separation gel into destaining buffer and let shake for 10 minutes
5. Repeat previous step at least twice again with fresh destaining buffer

In vivo

1. Bacterial cell culture

Materials & Equipment

- Bunsen burner
- Pipettes with peleus ball
- Micropipettes with sterile tips

Chemicals & consumables

- Culture tubes with metal caps
- Growth medium
- Autoclaved glass pipette tubes

Procedure

Starting culture: Under sterile conditions add about 5mL (two fingers high) of medium to a culture tube and insert the picked colony.

1. Cultivate the stock on agar plate or the like until colonies grow (incubation usually at 37°C)
2. Flame a glass pipette, open bottle of medium and flame mouth, measure out the amount you need to fill your tubes, flame cap and recap the bottle as quickly as possible.
3. Remove the tube cap, flame the top of the culture tube, pipette in 5 ml, flame the top of the tube, and cap it. Pick a single colony (to assure the cells are from the same single clonal population) and transfer it to the medium by tapping a small pipette tip (0.1µl tip held on a pipette) on the surface of the plate. Uncap a tube, flame the top, tip the tube to transfer cells from the pipette tip to the surface of the medium without touching the inside of the tube with the non-sterile portion of the pipette, flame, cap.
4. Pipette the desired amount of antibiotic into each tube along the wall. Do not put the non-sterile part of the pipette inside the tube and use a new tip for each tube.
5. Vortex each tube for 1-2 seconds to mix well.
6. Take the tubes to incubate in an incubator or warm room (usually at 37°C).
7. Wait overnight or until your cells have reached the desired concentration.[1]

1 http://openwetware.org/wiki/Bacterial_cell_culture

2. Cell counting/plating

Materials

- LB agar plate
- LB media
- Tubes

Procedure

1. Fill each tube used for the dilution with 90 µl of LB.
2. Add 10 µl of the sample to the first tube and mix.
3. From the first tube, remove 10 µl and mix it into second tube.
4. Repeat for the number of dilutions you wish to do (8 should be more than enough) [1].
5. Take 10 µl from each dilution and spot it on to the agar plate.
6. Allow droplet to dry and incubate.

The first dilutions will contain a thick lawn of cells and the last dilutions will contain no cells. There should be one drop which contains countable single colonies. From this, you can calculate the number of cells in the original sample. For example, if there 4 colonies on dilution 5, there are 4E4 cells/µl.[2]

2 http://openwetware.org/wiki/Bacterial_cell_culture

3. Chemically competent cells

The transformation of E. coli with plasmid DNA via heat shock transformation requires chemically competent cells.

Materials & equipment

- -80°C freezer
- Incubation shaker
- Centrifuge (cooling capabilities required!)
- Photometer
- Ice water bath

Chemicals & consumables

- Liquid nitrogen
- 50 mL Falcon tubes
- LB Medium
- Ice cold100mM CaCl2 solution& Mg^{2+}/ Ca^{2+} solution
- Ice cold 80% Glycerin

Mixtures

- Mg2+/Ca2+
    - 3.25 g $MgCl_2 \cdot 6\ H_2O$
    - 0.6 g $CaCl_2 \cdot 2\ H_2O$
    - 200 mL ddH_2O
    - Autoclave
- 100 mM Calcium Chloride
    - 2.95 g CaCl2·2H2O
    - 200 mL dH2O
    - Autoclave
      Note: You can also make a 1:10 dilution of the 1 M stock

Procedure

Day 1:

1. Grow Top10 / BL21 (DE3) overnight in 5 mL LB at 37°C.

Day 2:

1. Inoculate 100 mL LB with 1 mL of saturated overnight culture of *E. coli* cells
2. Incubate at 37°C and 150 rpm until OD_{600} 0.4-0.6 is reached (usually 2-3 h)
3. Incubate cells on ice for 5 min.

Note: After this point the cells should never touch anything warm—chill solutions, pipets, tubes, etc. beforehand.

4. Divide culture into 2 tubes with ~ 40 mL each
5. Centrifuge culture at 4°C and 3000 x g for 10 min
6. Gently resuspend each pellet with 15 mL cold Mg2+/Ca2+ solution

(Do not vortex!).

7. Incubate in an ice bath for 30 min
8. Centrifuge culture at 4°C and 3000 x g for 10 min
9. Resuspend each pellet with 1.6 mL of cold 100mM CaCl2 solution
10. Incubate in an ice bath for 20 min
11. Combine cells into one tube
12. Add 0.5 mL cold 80% glycerol and swirl to mix
13. Flash-freeze in liquid nitrogen as 100 µL aliquots
14. Store at -80°C.[3]

3 http://openwetware.org/wiki/Griffitts:Chemocompetent_Cells

4. Colony PCR

Materials & Equipment

- Thermocycler

Chemicals & consumables

- Sterile Eppendorf Tubes
- LB-agar plate with appropriate antibiotic
- Primers (usually VF2 and VR)
- Sterile pipet tips

Procedure

Colony PCR is a modified PCR program employed to verify transformation success by amplifying the insert or the vector construct used for transformation. This is necessary due to the fact that a transformation with the empty vector may also lead to the respective antibiotic resistance.

1. Reaction mixture 1x - (25 µL)
    - 12,5 µL 2x Taq MM
    - 0,5 µL VF2 (10 µM)
    - 0,5 µL VR (10 µM)
    - fill ddH_2O to 25 µL
2. Pick a colony with a sterile tip and suspend it in reaction mixture
3. Start PCR using the following program and 1X mix.
4. Run an agarose gel to determine the product length

Procedure

#	Temperature	Time
1	95 °C	00:02:00
2	95 °C	00:00:10
3	55 °C	00:00:30
4	72 °C	1 min/kbp
5	GO TO 2	REPEAT 30x
6	72 °C	1.5 min/kbp
7	4 °C	HOLD

5. Glycerol stock

In order to have a permanent culture of cells, glycerin stocks can be made.

Procedure

1. Add 200 µl of sterilized glycerin (50%) or DMSO to 800 µl cell culture and mix well.
2. Freeze the stock at -20 °C.

Also: Prepare 2-3 boxes with cut off tips for sterilization and store them separately for this purpose. The cut off tips allow an easy transfer of the viscose glycerol.

6. Heat Shock Transformation

Materials & equipment

- Heating bath
- Incubator

Chemicals & consumables

- Ice
- Pipets + steril tips
- LB medium
- LB-Agar-Plates + antibiotics

Procedure

1. Defrost stocks of competent cells (100 µL in 1.5 ml Eppendorf tube) on ice. This takes approximately 10 minutes.
2. Add DNA (2-6 µL) and incubate the suspension for 15 minutes on ice.
3. Heat shock is done by incubating cells for 45 seconds at 42°C. Afterwards, put samples back on ice for 2 min.
4. Add 1 mL LB medium and incubate at 37°C for 1 hour in order to obtain antibiotic resistance.
5. It might be useful to spin down cells at 5000 rpm for 5 min. Resuspend pellet in 100 µL LB.
6. Spread out

7. Protein Expression (T7-promoter system)

(If working with a different promoter system, use appropriate inducing substance and be careful to use appropriate strain)

Materials & equipment

- Incubation shaker
- Photometer

Chemicals & consumables

- *E. coli* BL21 DE3
- DYT Medium or LB
- IPTG
- 100 ml and 3 l flasks
- Ice

Procedure

(Choose volume appropriate to your desired amount of expressed protein)

1. Inoculation of 50 mL LB medium in a 100 mL flask with E. coli BL21 (DE3) strain transformed with desired plasmid
2. Incubation at 180 repulsion per minute (rpm) at 30°C to an OD_{600}= 4
3. Transfer starter culture into 1 L LB medium in a 3 L flask resulting in an OD_{600}= 0.2
4. Incubation to an OD_{600}= 0.6 at 180 rpm and 30°C.
5. Incubation for 15 minutes on ice.
6. Induction of protein expression with 20 µL of IPTG (stock concentration 1M).
7. Incubation of the cell suspension over night at 180 rpm at 30°C.

Materials

1. Buffers

Tris (1M, pH 7,5)

- 60.5g Tris base
- adjust pH to 7,5 using 5M HCl
- add ddH_2O to 500 mL

1x PBS

- 8.18 g NaCl (140 mM)
- 0.2 g KCl (2.7 mM)
- 1.77 g Na_2HPO_4 (10 mM)
- 0.24 g KH_2PO_4 (1.8 mM)
- Add. 1L ddH_2O
- Adjust to pH 7.4 using HCl

TE buffer

- 10 mM Tris, adjust to pH 8.0 using HCl
- 1 mM EDTA (Ethylenediaminetetraacetic acid)

50x TAE

- 242 g Tris base
- add 57.1mL glacial acetic acid
- 100 mL of 500 mM EDTA (pH 8.0) solution
- add up to 1 L with H_2O
- mix Tris with stir bar to dissolve in about 600 mL of ddH_2O
- add EDTA and Acetic Acid
- bring final volume to 1 L with ddH_2O
- store at room temperature

2. Media

1.7-DYT (recipe for 500 mL)

- 2.5 g NaCl
- 8.0 g tryptone
- 5.0 g yeast extract
- add 5 M NaOH to adjust the pH at 7.0 (few drops will be enough)

LB (recipe for 1000 mL)

- 10 g tryptone
- 5 g yeast extract
- 10 g NaCl
- add 5 M NaOH to adjust the pH at 7.0 (few drops will be enough)
- for plates: add 15g agar

SOB (recipe for 1000 mL)

- 0.186 g KCl
- 2.4 g $MgSO_4$
- 0.584 g NaCl
- 20 g tryptone
- 5 g yeast extract
- add 5 M NaOH to adjust the pH at 7.0 (few drops will be enough)

SOC (recipe for 1000 mL)

- 980 mL SOB
- 20 mL 1M Glucose

Autoclave all media after mixing!

3. Stock solutions

Antibiotics

Ampicillin

1. Mix
    - 4 g ampicillin (100 mg/mL)
    - add 40 mL ddH_2O
2. Sterile filtration
3. Aliquot in 1 mL stocks and store at -20°C
4. Use 1µL per 1mL medium

Chloramphenicol

1. Mix
    - 1g chloramphenicol
    - add 40mL ethanol
2. Aliquot in 1mL stocks and store at -20°C
3. Use 1µL per 1mL medium

Kanamycin

1. 1.Mix
    - 3 g kanamycin (75 mg/mL)
    - add 40mL ddH_2O
2. Sterile filtration
3. Aliquot in 1 mL stocks and store at -20°C
4. Use 1µL per 1mL medium

Induction chemicals

IPTG (Isopropyl-beta-D-thiogalactopyranoside)

1. Dissolve 238 mg IPTG in 10 mL water
2. Store in 1mL aliquots at -20°C

Recipes

9:25 Pfu MM (450 µL)

- 250 µL 10x Pfu buffer (BSA)
- 50 µL Pfu polymerase
- 50 µL dNTPs (10 mM)
- 50 µL MgCl2 (50 mM)
- 50 µL DMSO

2x Taq MM (1250 µL)

- 250 µL 10x Taq buffer
- 25 µL Taq polymerase
- 50 µL 50mM MgCl2
- 50 µL 10 mM dNTPs
- 75 µL DMSO
- 800 µL ddH2O

10x Pfu bufffer (100 mL)

- 200 mM Tris-HCl (pH 8.8 bei 25°C) 2.42 g
- 100 mM $(NH_4)_2SO_4$ 1.32 g
- 100 mM KCl 0.75 g
- 20 mM $MgSO_4$ x 7 H_2O 0.49 g
- 1 % Triton X-100 1 ml
- ddH_2O up to 100 ml
- Autoclaving/ sterile filtration after mixing.
- + 1 ml 100 mg/ml BSA (-> end conc. 1 mg/ml)

Recipe 10x Taq buffer (100 mL)

- - 500 mM KCl 3,7 g
- - 0,8 % Triton X-100 800 µL
- - 100 mM Tris-HCl (pH 8.8 bei 25°C) up to 100 ml
- Autoclaving/ sterile filtration after mixing.

References

1. J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular cloning : a laboratory manual*. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989).
2. A. Magnusdottir, I. Johansson, L. G. Dahlgren, P. Nordlund, H. Berglund, Enabling IMAC purification of low abundance recombinant proteins from E. coli lysates. *Nat Methods* **6**, 477-478 (2009).